Reply to 'Can we predict microbial keystones?'

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Samiran Banerjee, Klaus Schlaeppi and Marcel G.A. van der Heijden

In response to our recent Opinion article (Keystone taxa as drivers of microbiome structure and functioning. *Nature Reviews Microbiology* 16, 567–576 (2018)[1]Röttjersand Faust (Can we predict keystone taxa? *Nat. Rev. Microbiol*. XX XX 2018 doi. Xxx)[2] highlight an important discussion in microbial ecology: how accurately can keystones be predicted from co-occurrence networks? We agree with their claim that the identification of highly connected OTUs (hubs) in microbial networks does not necessarily reveal their role as keystones. In our Review article[1], we have extensively discussed that the identification of keystones by network-based scores must be complemented with empirical data to uncover their true importance. Therefore, we summarized keystones into two categories: those identified through computational inference (*keystone OTUs*) and those with explicit experimental evidence for their role in the microbiome (*keystone taxa*). While Röttjers and Faust only consider empirically established keystone taxa, our aim was to generate a candidate list for further validation but also to identify keystone OTUs, for which it might be a daunting task to collect enough empirical evidence to classify them as keystone taxa. Some microorganisms are uncultivable or current methods are not sensitive enough to culture them. Moreover, without an initial statistical screening for potential keystones, it may be nearly impossible to identify them in view of the enormous diversity and complexity in microbial communities (for example, almost any substrate on Earth is typically colonized by thousands of microbial taxa).

Röttjers and Faust also underpin that the 'conditionality' of co-occurrence networks often confounds the accurate identification of keystones. Indeed, network-based scores can be biased by habitat filtering and other environmental factors[3,4], and this was also extensively discussed in our article. However, we posit that keystone OTUs may also be important in the community in case they have been consistently identified across ecosystems. For example, we speculated in our article that mycorrhizae, an important and widespread group of beneficial fungi that contribute substantially to plant nutrition, might also be keystones. Our recent study found that the majority of keystone OTUs in the wheat-root microbiome were arbuscular mycorrhizal fungi[5]. Moreover, our study found some of the keystone OTUs were members of the *Dioszegia*, a genus that was identified as keystone taxa by Agler *et al.* (2016). The consistent identification of *Dioszegia* as keystone OTUs across studies not only highlights their importance in the plant microbiome but also indicates the usefulness of network scores for an initial identification of keystones, which can then be tested empirically. We would like to stress that owing to the ubiquitous nature and the enormous diversity of microorganisms, we expect that there are many more keystone OTUs than the 200 listed in Supplementary Table 1 (Ref. [1]).

In summary, we wish to thank Röttjers and Faust for reinforcing the importance of empirical evidence for confirming the role of keystone OTUs. We explicitly stated that network-mediated identification requires further proof and thus, we proposed several approaches, including metagenomics and metatranscriptomics. Recent *culturomics*[7] and *microbiome-on-a-chip*[8] approaches may also be useful. In science, experimental evidence often comes years after theoretical proposition. A classic example of this is '*comammox*' or complete nitrifiers, for which experimental validation[9] came a decade after it was originally postulated[10]. As

such, the identification of keystone OTUs will help to target key members of microbial communities.

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